REMARKS

Status of the claims

Claims 1-4 are pending in the application with claims 3 and 4 being withdrawn. Claims 1 and 2 are amended herein. Claim 1 has been amended to explicitly recite the feature that the LAK-induced sample is prepared by treating the lymphocyte fractions with the extract of *Lentinus edodes* mycelium in the absence of IL-2. Support for this amendment may be found at least in the working Examples of the specification. Claim 2 has been further amended to recite "raising the temperature of said suspension to sufficient temperature to inactivate the enzyme(s)." Support for this amendment may be found in the specification at least at page 9, 2nd from final line, or page 17, lines 1-3, which state that one purpose of heating the extract is to inactivate the enzyme. No new matter has been added with these amendments. As such, entry thereof is respectfully requested.

Rejection under 35 U.S.C.§112, 1st paragraph

The Examiner rejects claim 2 under 35 U.S.C.§112, 1st paragraph for the alleged recitation of new matter. The Examiner asserts that there is no support in the specification for the recited temperature range of "80-100°C." Claim 2 has been amended, as indicated above, to recite "raising the temperature of said suspension to sufficient temperature to inactivate the enzyme(s)." While the specification does not provide *ispsis verbis* support for recitation of "sufficient temperature", Applicants believe that specification clearly supports that heating to a sufficient temperature to inactivate the enzyme(s) is the intended feature of the inventors and one skilled in the art would readily know that this was intended and how to determine whether the enzyme(s) is inactivated. For example, the detailed description of the invention states that the suspension should be heated to 95°C to inactivate the enzyme and in the Examples the suspension was heated to 90°C. Thus, it is clear that a single temperature is not required. In addition, the specification explicitly recites that one purpose of the heating step is to inactivate the enzymes. As such, entry of the amendment is respectfully requested.

Rejection under 35 U.S.C.§102(b)

Claim 1 has been rejected under 35 U.S.C.§102(b) as being anticipated by Tani et al. Tani et al. is asserted to teach that "lentinan", which is a polysaccharide extract of *Lentinus edodes*, enhances LAK activity of PBMC. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

It is clear from a full reading of Tani et al. that the lentinan of the reference only has activity on LAK cells in the presence of IL-2. For example, in Table 1 of the reference lentinan alone had no effect on the cytotoxicity against autologous tumor cells. The extract of the invention, on the other hand, activates LAK cells in the absence of IL-2. Claim 1 has been amended to recite that the present invention is directed to a method for determining whether an extract of *Lentinus edodes* mycelium *in vitro*, in the absence of IL-2 has a LAK activity-enhancing effect and that the extract is prepared in the absence of IL-2. There is no disclosure of suggestion in Tani et al. of an extract of *Lentinus edodes* mycelium that has a LAK activity-enhancing effect in the absence of IL-2. As such, the instant invention is not anticipated by Tani et al. and withdrawal of the rejection is respectfully requested.

Claim 1 has been further rejected as being anticipated by Li et al. As with Tani et al., Li et al. also teaches the combined exposure of the PMBC to both lentinan and IL-2. As such, for the reasons discussed above regarding Tani et al., the instant invention is not anticipated by Li et al. and withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C.§103

Claims 1-2 have been rejected under 35 U.S.C.§103 as being obvious over Yamamoto et al. in view of Liu et al. Yamamoto et al. is asserted to differ from the instant invention in failing to teach that an extract of *Lentinus edodes* mycelia enhances cytotoxic activity of NK cells stimulated with IL-2.

The new reference of Liu et al. is relied on for teaching that a polypeptide-polysaccharide complex from *Lentinus edodes* stimulates IL-2 production in PMBC. The Examiner appears to assert that it would be obvious to also use the extract of Yamamoto et al. on

lymphocyte cells with a reasonable expectation of successful activation of the LAK cells because Liu et al. demonstrate that *Lentinus edodes* stimulates IL-2 production, and presumably the IL-2 would then activate the LAK cells present. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

The examiner describes, referring to page 196, sections 3.1 and 3.2 of Liu et al., that the reference teaches that production of IL-2 in PBMC treated with a crude extract of *Lentinua edodes* mycelia was measured by the levels of mRNA and protein *in vitro* and *in vivo.* However, Liu et al. do not describe any *in vivo* experiments. Section 3.1 only describes experimental data using "murine spleen mononuclear cells which were cultured with different concentrations of LE" and "the peritoneal exudate macrophages which were cultured in the presence of 25, 50, 100 microgram/ml of LE". Similarly, Section 3.2 only describes the experimental data using "human PBMC which were cultured with different concentrations of LE." Thus, the authors of Liu et al. did not examine *in vivo* LAK activity enhancing effect of the extract of *Lentinus* edodes mycelia when the extract is administered to mouse or human *in vivo.*

The present invention demonstrates for the first time that an extract of *Lentinus edodes* mycelium, i.e. the extract made in accordance with the method described in the specification and recited in the claims, has the ability to activate LAK cells *in vivo* based on the fact that the *in vitro* LAK activity enhancing effect of the extract is parallel to that *in vivo.*

The invention of claim 1 is directed to a method for determining whether an extract of *Lentinus edodes* mycelium *in vitro*, in the absence of IL-2, has a LAK activity-enhancing effect suitable for a subject, comprising the steps of:

(a) isolating peripheral blood from the subject to prepare lymphocyte fractions,

(b) preparing a LAK-induced sample by treating the lymphocyte fractions with the extract of *Lentinus edodes* mycelium in the absence of IL-2, and preparing a control sample in the absence of the extract of *Lentinus edodes* mycelium, and

(c) measuring and comparing the LAK activity of the LAK- induced sample and the control sample to determine *in vitro* whether the extract of *Lentinus edodes* mycelium has a LAK activity-enhancing effect suitable for the subject.

Thus, the invention of claim 1, is directed to a method for determining *in vitro* whether an extract of *Lentinus edodes* mycelium will have suitable *in vivo* LAK enhancing activity to make the extract useful for LAK-therapy in a patient.

Similarly, the present specification states at page 6, line 23 through page 7, line 1 that "the *in vivo* cytotoxicity, which is exerted by the direct administration of an antitumor or anticancer agent, especially a LAK activity enhancer containing *Lentinus* edodes mycelium extract, has a positive correlation with the cytotoxicity which is exerted when lymphocytes prepared from a subject are activated with the LAK activity enhancer" (Page 6. line 23 - page 7, line 1). This finding is supported by Example 2, which describes the positive correlation between the *in vitro* and *in Vivo* LAK activity enhancing effects of the extract (See, Figure 1).

Thus, the present invention provides, for the first time, a means for solving a significant problem associated with LAK therapy. That is to say, if the extract of the invention can be administered to a patient after confirmation of whether LAK cells from the patient are actually activated by the addition of the extract with an *in vitro* test, the fundamental problem that "LAK therapy exerts different effects in different individuals and sometimes has almost no effect" (see page 3, lines 6-7 of the specification) can be overcome. Since the extract of the present invention does not give rise to side effects in humans and the raw material of the extract is much cheaper than IL-2, the extract of the invention provides a novel alternative to the treatment of a disease mediated by activated LAK cells (i.e. LAK therapy) .

The method of the invention is useful in determining whether an extract of the invention actually has a LAK activity enhancing effect for a patient before conducting the LAK therapy.

Those ordinary skill in the art could not have arrived at such a feature of the invention, nor conceived of the concept of the present invention based on the teachings of Yamamoto et al. and Liu et al., since neither reference contains a description or suggestion of activating LAK cells using the extract of *Lentinus edodes* mycelium of the invention both *in* vitro and *in vivo* and more importantly of the correlation between the *in vitro* LAK activity enhancing effect of the extract and the *in vivo* LAIC activity enhancing effect.

As such, the present invention is not obvious from Yamamoto et al. in view of Liu et al. and withdrawal of the rejection is respectfully requested.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

If the Examiner has any questions concerning this application, the Examiner is requested to contact MaryAnne Armstrong, Ph.D., Reg. No. 40,069 at the telephone number of (703) 205-8000.

Dated: APR 20 2006

Respectfully submitted,

By _____

MaryAnne Armstrong, Ph.D.
Registration No.: 40,069
BIRCH, STEWART, KOLASCH & BIRCH, LLP
8110 Gatehouse Road
Suite 100 East
P.O. Box 747
Falls Church, Virginia 22040-0747
(703) 205-8000
Attorney for Applicant